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5-Aminolevulinic Acid (ALA)-Induced Protoporphyrin IX Fluorescence and Photodynamic Effects in the Rat Bladder: An In Vivo Study Comparing Oral and Intravesical ALA Administration

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Background and Objective: **Photodynamic therapy (PDT) using 5-aminolevulinic acid (ALA)-induced protoporphyrin IX (PpIX) for sensitization is a promising treatment for carcinoma in situ and diffuse premalignant changes of the bladder. We studied the biodistribution of PpIX in a range of tissues with oral and intravesical routes of administration of ALA and compared the photodynamic effects on bladder and skin.**
Study Design/Materials and Methods: **Normal Wistar rats were given oral or intravesical ALA and PpIX levels in the liver, kidney, skin, and bladder measured by fluorescence microscopy on tissue sections. At the time of maximum PpIX levels, the bladder and skin on the back were illuminated with light at 630 nm and the PDT effects compared.**
Results: **PpIX fluorescence in the urothelium after 200 mg/kg given intravesically was comparable to that found after 100 mg/kg orally. The ratio of PpIX levels between the urothelium and the underlying muscle was the same for both routes of administration, although there appeared to be more selectivity of urothelial PDT necrosis after intravesical administration. Skin photosensitization was greater after oral ALA, the epidermal PpIX level being three times higher than after intravesical administration for comparable urothelial levels and the PDT effect being more marked.**
Conclusions: **Intravesical instillation is preferable to oral administration of ALA for PDT ablation of the urothelium of the rat bladder without damage to the underlying tissue layers and for minimizing skin photosensitivity. The technique is now ready for clinical trials. Lasers Surg. Medicine 20:254–264, 1997.**

Key words: 5-Aminolevulinic acid (ALA); bladder cancer; fluorescence microscopy; photodynamic therapy; skin photosensitization; intravesical therapy

INTRODUCTION

Photodynamic therapy (PDT) is a modality receiving increasing attention in many medical specialties for the treatment of early malignancies [1,2]. By the combined action of a photosensitizer and light illumination in the presence of

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tissue oxygen, photochemical cellular destruction can be achieved if a sufficient amount of photosensitizer is present in the tissue and light of appropriate wavelength and energy is delivered [3]. Hematoporphyrin derivative (HpD) or its purified ether/ester (Photofrin) is the most frequently used photosensitizer in current PDT studies, but the prolonged cutaneous photosensitization, which lasts for 4–6 weeks, is an obvious disadvantage. Research on developing new photosensitizers is being widely pursued, but the problem of cutaneous photosensitivity remains. An alternative approach is to use local administration such as intratumoral injection or intravesical administration, which has met with varying degrees of success [4–6].

The use of 5-aminolevulinic acid (ALA), a precursor of protoporphyrin IX (PpIX), has been extensively studied in vitro [7], in vivo [8,9], and in clinical trials [10–12] in recent years and is a promising new approach for PDT. ALA is a naturally occurring photoinactive intermediary in the cellular biosynthetic pathway for heme. In this chain reaction, ALA is converted in small amounts to coproporphyrin and uroporphyrin, but mainly to PpIX, the predominant porphyrin species responsible for photosensitization [10]. Since the conversion of PpIX to heme is the rate-limiting step and is controlled by ferrochelatase and tissue iron, bypassing the normal regulatory feedback mechanism with excess exogenous ALA results in temporary accumulation of PpIX [10]. By this means, photodynamic therapy has been successfully undertaken for the treatment of skin cancer [10,11] and other human malignancies [12,13]. The use of ALA/PpIX for PDT of dysplastic lesions in hollow organs such as the bladder has attracted much interest, since the risk of significant systemic side effects should be minimal [12,13]. As previous urological experience with HpD-based PDT for bladder cancer treatment showed a high incidence of complications (particularly scarring of detrusor muscle and contracted bladders) [14], we studied the feasibility of instilling ALA directly into the bladder as a means of achieving selective urothelial sensitization compared with the underlying muscle [6] and assessed the photodynamic responses after light illumination [15]. Further to our previous studies, in the present study, we compared the intensity of PpIX fluorescence in various organs at different times after giving either oral or intravesical ALA and evaluated the difference of photodynamic effects on urinary bladder and skin using the two routes of sensitization.

MATERIALS AND METHODS

Animals and Anesthesia

Female Wistar rats, weighing 100–160 g, were used in this study. The ALA solution was administered by gastric gavage to rats anesthetized with a mixture of Halothane and O_2 (volume ratio 1:2). However, for longer procedures such as transurethral instillation of ALA and laser treatment, general anesthesia was used with intramuscular injection of 0.1 ml/kg Hypnorm (fentanyl and fluanisone, Jansen Pharmaceuticals, Grove, UK) and 1 mg/kg diazepam.

Photosensitizer

ALA (ALA.HCl) was obtained as a 98% pure powder from DUSA Pharmaceuticals, (New York, NY). As in our previous study [15], we used 10% ALA solution (100 mg/ml) at pH 5.5 for bladder instillation. This was prepared immediately prior to administration by buffering the ALA solution with saturated sodium bicarbonate.

ALA Administration

Bladder instillation (BI) of ALA was accomplished by infusing a 10% solution at the dose of 200 mg/kg (0.2 ml/100 gm body weight) through an 18-gauge Teflon catheter, which had been inserted transurethrally and was kept in the bladder for 2 hours while the rats remained under general anesthesia. Throughout the text, instillation time refers to the time ALA was kept in the bladder with the animal under general anesthesia, whereas retention time refers to the sum of instillation and postinstillation times until the animals were killed (for the biodistribution study), or treated (to look at the photodynamic effect).

Oral administration (Oral) of ALA was undertaken by giving ALA solutions at doses of 100, 200, and 400 mg/kg body weight through a bulb-tip gavage needle into the stomach under inhalation anesthesia. As rats are incapable of vomiting or regurgitation, all animals ingested the full delivered dose. All the rats resumed normal activity within 5 minutes of terminating the anesthetic. The oral doses used were those found in preliminary studies to give urothelial levels of PpIX comparable to those found in our previous study using intravesical ALA.

Fluorescence Microscopy

For each ALA dose and route of administration, 2–3 rats were studied. The control group consisted of two rats receiving nothing and two others with bladder instillation of 0.9% normal saline (0.24–0.3 ml, depending on body weight). The rats were killed and specimens from the urinary bladder, liver, kidney, and back skin were collected 1, 2, 3, 4, 5, 7, 9, and 24 hours after administration of ALA. The tissues harvested were initially placed in precooled isopentane (BDH Chemicals, Poole UK) and then transferred to liquid nitrogen before being processed to thin frozen sections (10 μm) for fluorescence imaging.

Microscopic imaging and quantification of ALA-induced PpIX fluorescence was done with an inverted phase contrast microscope (Olympus IMT-2) attached to a slow-scan, charge coupled device (CCD) camera (Wright Instruments, London, UK, model 1, 385 × 578 pixels). The setup of this highly sensitive photometric system has been described in previous studies on fluorescence imaging after administration of photosensitising agents [9,16]. The slow-scan CCD system permitted highly reproducible signal calibration, which was checked using a thin film on a glass slide under a coverslip of a mounting medium (Fluoromount, BDH Poole, UK) containing aluminium phthalocyanine at a concentration of 10 μg/ml. In brief, an 8 mW helium neon laser (632.8 nm) was used to excite the PpIX and the emitted fluorescence was detected between 660 and 710 nm, using a combination of bandpass and longpass filters. This excitation wavelength is ideal for PpIX but less so for uro- or coproporphyrins, which show peak absorption at slightly shorter wavelengths nearer 620nm [17]. The fluorescence signals were processed by an IBM personal computer into a falsely color-coded image depicting the mean signal per pixel. The software also allowed fluorescence data to be quantified digitally over defined areas from which the mean counts per pixel could be calculated. After fluorescence imaging, the sections were fixed in formaldehyde and stained with hematoxylin and eosin for histologic matching.

The fluorescence intensity of specific tissue layers measured in the bladder and skin (urothelium, lamina propria, muscularis propria, skin epidermis, dermis, and hair follicle) was the average measurement from > 20 representative blocks (minimum 10×10 pixels) with correction for background autofluorescence. However, for sections of the solid organs (liver, kidney, and abdominal muscle), the fluorescence quantification profile was the average of 10–12 representative blocks (minimum 350×500 pixels) taken from various areas. No correction for background autofluorescence was applied for these organs.

Photodynamic Therapy

The control group consisted of three rats receiving laser illumination without ALA sensitization. The experimental groups were stratified into Group A: Intravesical ALA (200 mg / kg), and 50J light; Group B: Oral ALA (100 mg/kg) and 50J light; Group C: Oral ALA (200 mg/kg) and 25J light; Group D: Oral ALA (200 mg/kg) and 50J light, and Group E: Oral ALA (400 mg/kg) with 50J light dose. The light source was a copper vapor-pumped dye laser (Oxford Lasers) tuned to 630 nm, the beam being delivered through a 200-μm silicon-coated quartz fibre. At the optimal time after sensitization (5 hours with intravesical, 4 hours with 200 and 400 mg/kg oral, and 3 hours with 100 mg/kg oral ALA), a small abdominal incision was made under general anesthesia to expose the urinary bladder. The bladder was then eased upward and placed on the abdomen, emptied of its urinary contents with a transurethral cannula, and filled with 0.3 ml of 10% intralipid solution to ensure homogenous light scattering. At this volume the bladder was approximately spherical in shape, which further ensured isotropic light distribution. The tip of a bare laser fiber (without cladding) was placed ~ 3 mm from the top of the bladder after insertion through the dome. No intralipid leakage was seen on puncturing the bladder as the fenestration sealed tightly around the fiber. The power from the fibre tip was calibrated before and after each treatment and was set at 100 mW for 250 or 500 seconds giving a total light dose of 25 or 50J, respectively, per treatment. Following PDT, animals were killed at 2, 3, and 7 days and the bladder sectioned and stained for histological assessment. Four rats, two with intravesical (200 mg/kg) and two with oral ALA (400 mg/kg), were treated at a light dose of 50J and were kept alive for 6 months after PDT for long-term comparisons. These specimens were stained with Van Gieson for assessment of collagen fibrils in the bladder wall.

Eight rats (2 without drug, 2 with intravesical ALA, 2 with 200 mg/kg oral, and 2 with 100 mg/kg oral ALA) were investigated for the skin photosensitivity study. All were treated 3 hours after receiving ALA. The fur overlying the lower abdomen was shaved and the exposed skin was

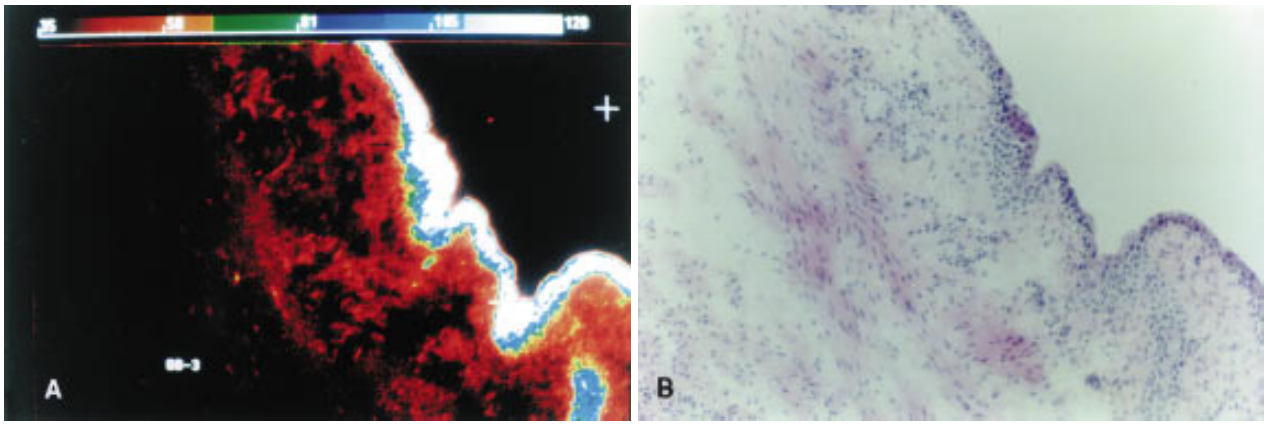

Fig. 1. **A.** Fluorescence microscopy of rat urinary bladder 5 hours after instillation of ALA showing higher PpIX (White) intensity in urothelium than in the underlying layers (Red). The fluorescence scale is shown in the color bar at the top. **B.** Corresponding histological section H&E stain (×150).

illuminated with red light (630 nm) through a 200-μm laser fiber placed 1 cm above the surface at a power of 100 mW (power density 100 mW/cm^2) for 500 seconds (50J/cm^2). The animals were killed 3 days after light illumination and the treated areas examined macroscopically and sectioned and stained with hematoxylin and eosin (H & E) for histologic evaluation.

RESULTS

Fluorescence Microscopy

The highest levels of PpIX fluorescence were present in the urothelium (Fig. 1A,B). Levels rose faster following oral administration of ALA (Fig. 2). At the higher oral doses (200 and 400 mg/kg), fluorescence in the urothelium peaked at 6–7 hours, but at 100 mg/kg, it was at 3 hours. With bladder instillation of 200 mg/kg, the peak urothelial level occurred between 4 and 7 hours, at which time it was similar to that found 3 hours after 100 mg/kg orally. It was notable that bladder instillation gave a more sustained level of PpIX in the urothelium, whereas the level fluctuated more after oral administration. Although the peak urothelial intensity was generally higher in the oral groups, the ratio between the level in the urothelium and in the underlying layers remained similar as the fluorescence signals in the lamina propria and muscle were also higher with oral administration (Fig. 3). The results in the liver are shown in Figure 4. At the early times following oral ALA, fluorescence in the liver was much higher than after bladder instillation. The signal observed probably arises from protoporphyrin, uroporphyrin, and coproporphyrin (see Discussion). However, at longer times, the differential declined and was virtually abolished after 7 hours. The fluorescence levels in the renal cortex resembled those of liver, but wide fluctuations in the readings prevented any clear pattern being identified (Fig. 5). It was notable that readings of fluorescence levels at time zero, which indicated the baseline autofluorescence in the liver and kidneys without ALA administration, were much higher than in the other organs measured, presumably due to the presence of endogenous porphyrins. Despite the lack of a clear pattern in terms of porphyrin pharmacokinetics, hepatic fluorescence signals returned to baseline values by 24 hours after ALA administration.

PpIX fluorescence levels in the epidermal and dermal layers of the skin on the back are shown in Figures 6 and 7. In each layer, signals from the oral groups were generally higher than those from the BI group. At an oral dose of 100 mg/kg, which gave a peak urothelial PpIX level similar to that from 200 mg/kg given by BI, PpIX in the epidermis was twice as high as in the BI group. Intravesical instillation also resulted in less PpIX buildup in the hair follicles.

Photodynamic Therapy

Macroscopic findings. Control bladders that underwent light illumination only showed no evidence of tissue injury. Under the most severe conditions (Group E, 400 mg/kg oral ALA and 50J light), 3 of the 12 rats died within 48 hours. Others in the group that survived the early stages

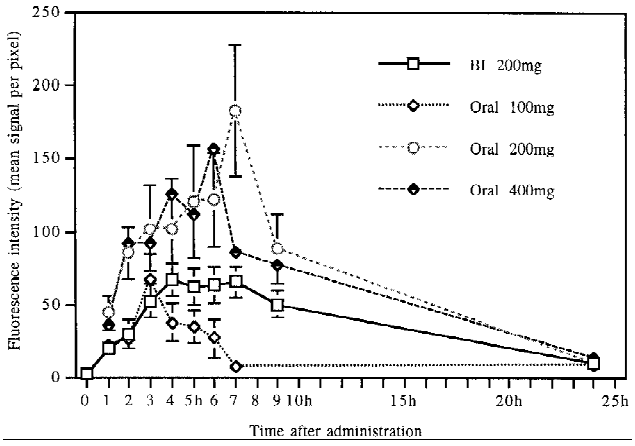

Fig. 2. Plot of PpIX fluorescence intensity in the urothelium after oral or intravesical ALA.

were physically unwell until 7 days after PDT. The perioperative findings of rats in Group D (200 mg/kg oral ALA and 50J light) were similar to Group E, and two died within 48 hours of PDT. However, with a reduced light dose: Group C (200 mg/kg oral ALA and 25J light), or reduced ALA dose: Group B (100 mg/kg oral ALA and 50J light), no perioperative mortality was seen. Similarly, for intravesically sensitized rats in Group A, no PDT-associated death or perioperative distress was observed. On necropsy of the rats that died within 48 hours of PDT (Group D and E), the most probable cause of death was extravasation of urine with possible peritonitis as accumulation of turbid fluid in the abdomen was a consistent finding. The cause of urinary extravasation remains uncertain, although there is the possibility of leakage through the site of fibre puncture, which could have become an established perforation after PDT.

Whatever the dose of oral ALA, 48 hours after treatment the bladder looked inflamed, puffy, and edematous. Only the five animals described above showed evidence of perforation, although a perivesical fatty tissue reaction was prominent in most. The bladders receiving 50J of light contained some tissue debris and were particularly thin and flaccid on filling with formalin. The lower light dose of 25J provoked less tissue reaction. By the seventh day, perivesical fatty inflammation was subsiding, although was not completely resolved. Animals with intravesical ALA (Group A), however, demonstrated much milder changes macroscopically at these times after PDT. Six months following PDT, the appearance of bladders and the surrounding tissue in animals that had been sensitized with oral or intravesical ALA were macroscopically indistinguishable from those of control rats, even though the ones given oral ALA had received the highest drug and light doses.

Microscopic findings. Histologically, no evidence of urothelial damage was discovered in rats receiving light but no ALA. However, mild inflammatory cell infiltration in the serosa and outer layer of muscularis propria was seen, probably due to handling. The bladder lesions 2–3 days after PDT were more severe in the oral ALA group than in the instillation group. Those treated with 50J showed full thickness tissue destruction with fibrinoid necrosis of arterioles in most parts of the bladder (Fig. 8). At 25J, the urothelium was ablated with preservation of part of the underlying structures. However, muscle damage of some degree seemed inevitable. Interestingly, little urothelial destruction was observed in Group B, although the fluorescence intensity at 3 hours was as high as that at 5 hours in Group A. Despite nearly complete regeneration of damaged urothelium by 7 days after PDT, subepithelial fibroblast infiltration was seen, especially in the lamina propria. In contrast, the histological changes found with intravesical ALA showed less prominent but more selective urothelial ablation without lamina propria or muscle damage (Fig. 9), even though they were treated with 50J. By the 7th day, bladder epithelium had regenerated and fibroblast infiltration was hardly seen in either lamina propria or muscle. The findings 6 months after PDT revealed significantly more collagen fibrils in both the lamina propria and the muscularis propria of the oral group compared with the intravesical group (Figure 10). The microscopic findings are summarized in Table 1.

Skin. Macroscopically, no visible lesion was seen in the control and intravesically sensitized rats. Those having oral ALA showed brownish discoloration of the skin especially in the centre where the light was greater. Microscopically, control rats treated with light alone showed normal skin architecture (Fig. 11A). In the ALA instillation group, focal epidermolysis with very mild keratolysis was observed. The epidermis and dermis were normal, whereas the loose connective tissue layer showed mild infiltration of inflammatory cells (Fig. 11B). In contrast, the skin histology of those receiving oral ALA demonstrated extensive destruction of the epidermis and dermis. There was intensive inflammatory cell infil-

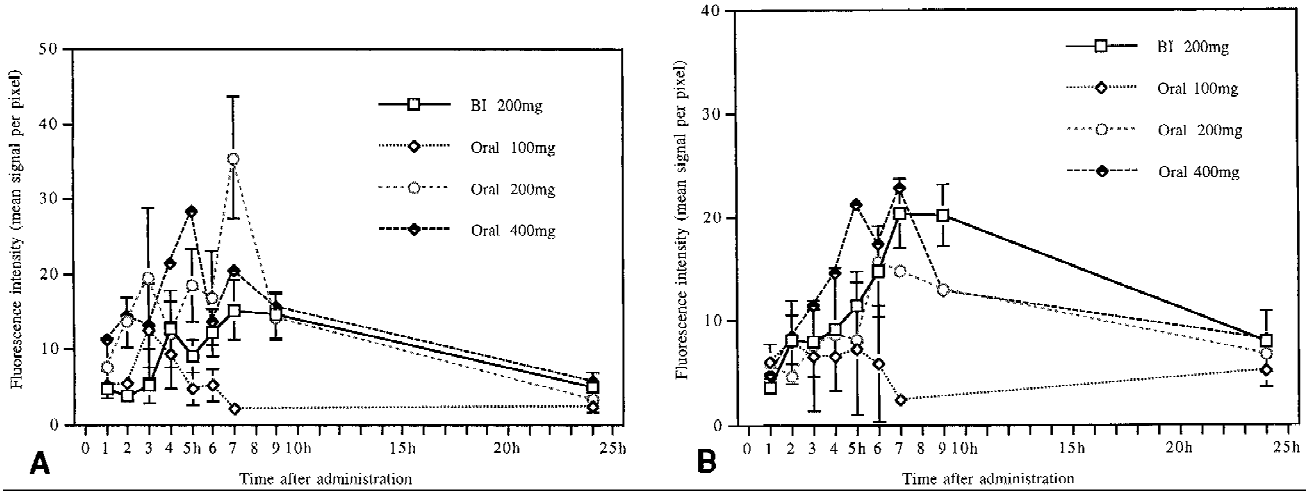

Fig. 3. **A.** Plot of PpIX fluorescence intensity in the lamina propria. **B.** Muscularis propria after giving ALA via the oral or intravesical route.

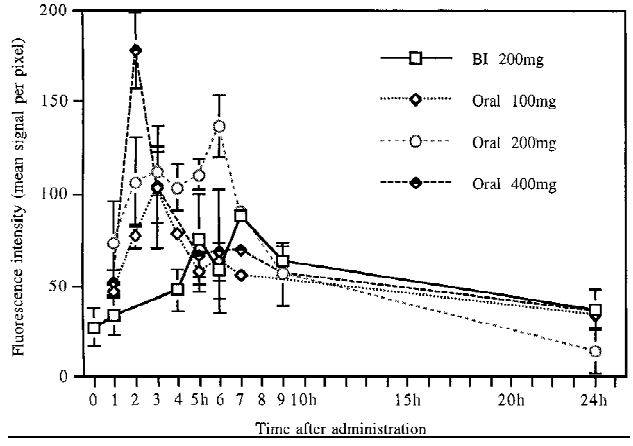

Fig. 4. Plot of PpIX fluorescence intensity in the liver after giving ALA via the oral or intravesical route.

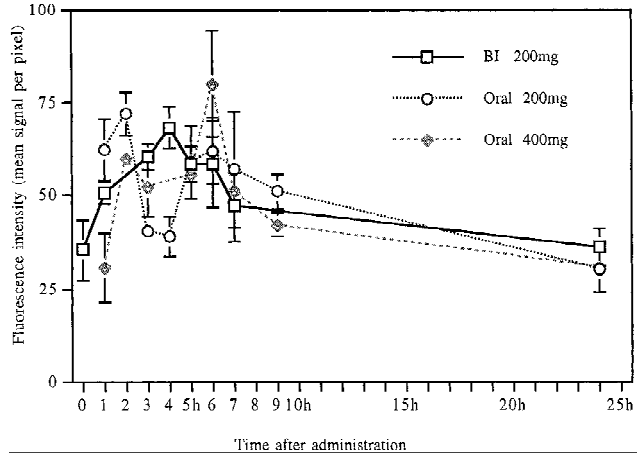

Fig. 5. Plot of PpIX fluorescence intensity in the kidney showing a fluctuating pattern of PpIX after giving ALA via the oral or intravesical route.

tration where the dermis meets the underlying connective tissue layer (Fig. 11C). The microscopic findings were more marked in those treated with 200 mg/kg compared with those given 100 mg/kg orally.

DISCUSSION

Although PDT has the potential for treating bladder tumors and some of the preliminary results using HpD as a sensitizer seemed encouraging [18–20], its universal acceptance is unlikely unless the concerns of detrusor muscle damage [14] and prolonged skin photosensitization [21] can be satisfactorily resolved. In our previous study using 10% ALA solution at pH 5.5 administered intravesically and treating the bladder with a light dose of 50J (630 nm), we were able to induce homogenous urothelial ablation with only negligible damage to the underlying layers [15]. The selective necrosis of the transitional cell layer produced with ALA induced PpIX is therefore relatively mild and well tolerated. Although the duration of skin photosensitization with ALA is only 1–2 days [12], measures that could reduce its severity would be welcome. From this study, intravesical administration is the preferred route for treating the bladder as for comparable levels of urothelial PpIX, there was less full thickness bladder damage and less skin photosensitivity.

For any substance to be efficiently transported into the urothelium after intravesical administration, it should have a low molecular

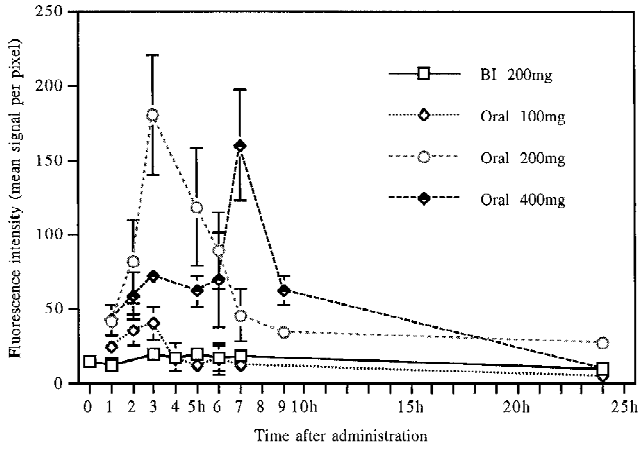

Fig. 6. PpIX fluorescence intensity in the epidermis of back skin after oral or intravesical ALA.

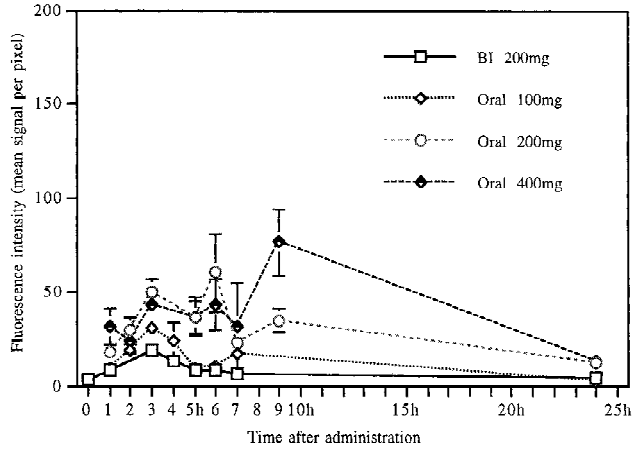

Fig. 7. PpIX fluorescence intensity in the dermis of back skin after oral or intravesical ALA.

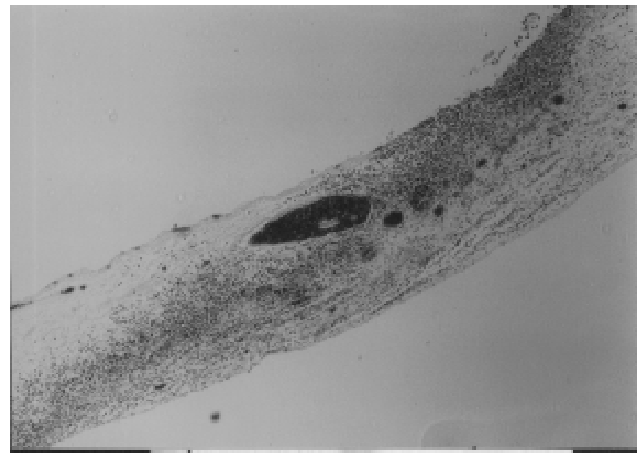

Fig. 8. Microscopic picture 48 hours after PDT showing full thickness bladder damage with fibrinoid necrosis of vessels and prominent inflammatory cell infiltration. No evidence of selectivity between layers is seen (50J, 4 h after 400 mg/kg orally) (H&E stain, ×60).

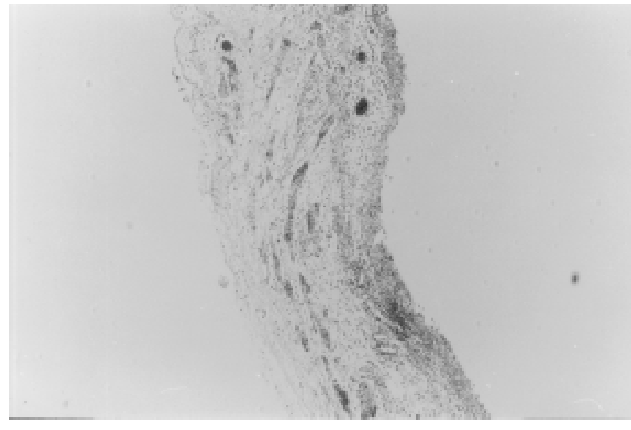

Fig. 9. Microscopic picture of bladder 48 hours after PDT showing selective destruction of urothelium and relative preservation of the muscularis propria (50J, 5 h after bladder instillation of 200 mg/kg) (H&E stain, ×60).

weight (<200), be watersoluble, and yet be sufficiently lipophilic for binding with plasma membranes [22]. The biochemical properties of ALA (amphiphilic with molecular weight of 131) fulfil these criteria. Amino acids of similar molecular weight to ALA also have been shown to diffuse through the urothelium over a concentration gradient [23]. The buildup of PpIX fluorescence in the urothelium after instilling the ALA solution is good evidence to support the permeability of the urothelium to ALA [6]. After penetrating the superficial umbrella cells, it may take some time to diffuse into deeper transitional cell layers and to be converted to PpIX. The duration of bladder instillation is important as there is continuous PpIX generation in the cells, so the tissue level of PpIX can build up through the constant contact of the urothelium with ALA. The relatively constant level of urothelial PpIX 4–7 hours after bladder instillation is another advantage over oral administration as it makes photodynamic effects in the bladder more uniform and predictable. Systemic administration, however, provides ALA for PpIX biosynthesis through the vasculature, so most ALA would be expected in the best vascularised layers, the urothelium, and lamina propria. Nevertheless, conversion to PpIX may still be more efficient in the urothelium due to the greater metabolic activity of these cells, as was shown by Loh et al. [24]. From our previous report [24], peak PpIX levels in the epithelium here reached earlier at lower ALA doses (3 h at 100 mg/kg, 7–8h at 200 and 400 mg/kg). It is also noteworthy that 400 mg/kg orally produced no higher peak PpIX in the urothelium than 200 mg/

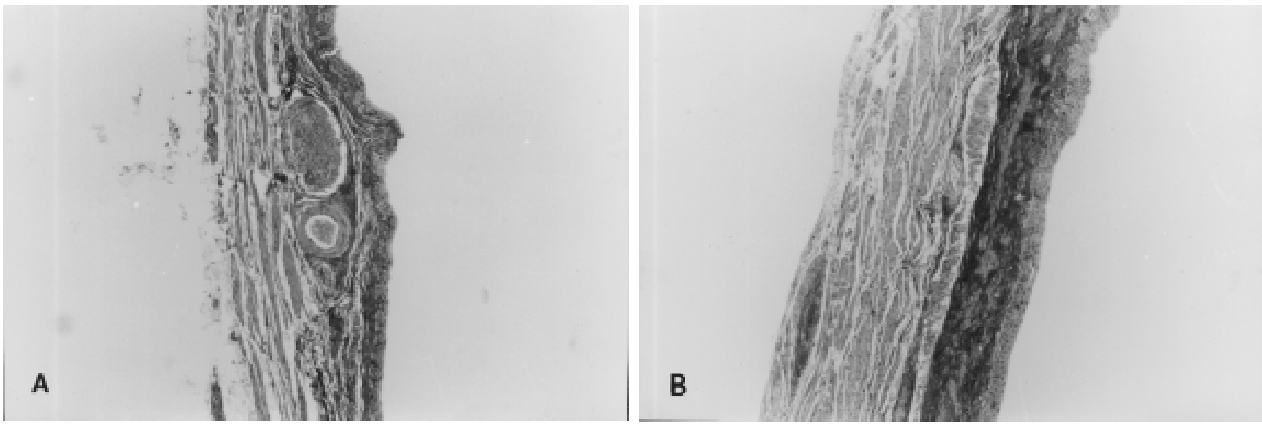

Fig. 10. Microscopic finding of bladder 6 months after PDT showing (**A**) slightly increased collagen fibrils in the lamina propria (200 mg/kg ALA, intravesical, 50J). **B** marked deposit of collagen in the lamina propria (400 mg/kg ALA orally) (HVG stain × 100).

TABLE 1. Histological Changes of Bladder Wall (2–3, 7, and 180 days) after PDT in Different Experimental Groups*

Group	Urothelium damage	Oedema L.P.[a]	Oedema M.P.[b]	Necrosis of urothelium and L.P.[a]	Muscle necrosis	Inflamm. cell infiltration L.P.[a]	Inflamm. cell infiltration M.P.[b]	Inflamm. cell infiltration Serosa	Fibroblast L.P.[a]	Fibroblast M.P.[b]	Fibroblast Serosa
Control (light illumination only)											
	–	–	–	–	–	±	–	±	–	–	–
A (BI[c] 200 mg/kg, 50J)											
2–3d	diffuse	++	+	++	+	++	++	+	–	–	–
7d	–	–	–	–	–	+	±	–	+	±	–
180d	–	–	–	–	–-	–	–	–	++	+	+
B (Oral 100 mg/kg, 50J)											
2–3d	patchy	–	–	±	++	++	+	+	++	+	±
7d	–	–	–	–	+	++	+	+	+	+	+
C (Oral 200 mg/kg, 25J)											
2–3d	extensive	++	+	+++	++	+	+	+	–	+	–
7d	–	–	–	–	±	+	±	–	+	±	±
D (Oral 200 mg/kg, 50J)											
2–3d	extensive	++	+	+++	+++	++	+	+	++	++	+
7d	patchy	–	–	+	±	+	+	±	++	+	+
E (Oral 400 mg/kg, 50J)											
2–3d	extensive	++	++	+++	+++	+	+	+	++	++	+
7d	patchy	±	±	+	±	+	+	±	++	+	+
180d	–	–	–	–	–	–	–	–	+++	++	++

*Treatment parameters: ALA concentration, light dose. Grade of histological changes: –: none, ±: minimal, +: mild; + +: moderate; + + +: severe.
[a]Lamina propria.
[b]Muscularis propria.
[c]Bladder instillation.

kg. It is probable that above 200 mg/kg, the available ALA saturates the synthetic capacity of the urothelium so further increases of PpIX levels are impossible.

This report has shown better selectivity of PDT necrosis of the urothelium compared with the underlying layers using intravesical ALA. This was unexpected as the fluorescence microscopy studies showed that the selectivity of PpIX distribution between layers was the same for both routes of administration. This may be because with bladder instillation, the PpIX is located in

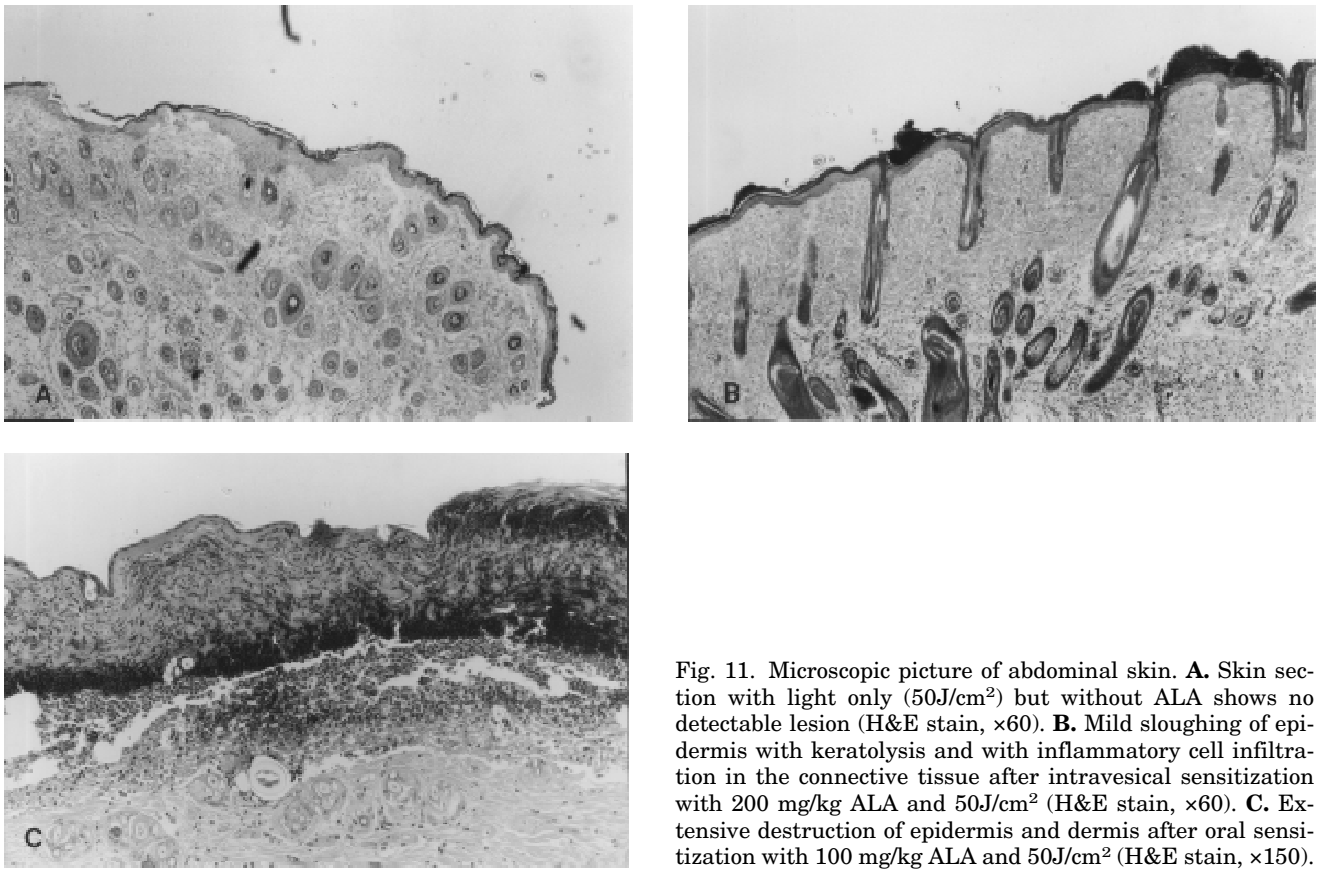

Fig. 11. Microscopic picture of abdominal skin. **A.** Skin section with light only (50J/cm^2) but without ALA shows no detectable lesion (H&E stain, ×60). **B.** Mild sloughing of epidermis with keratolysis and with inflammatory cell infiltration in the connective tissue after intravesical sensitization with 200 mg/kg ALA and 50J/cm^2 (H&E stain, ×60). **C.** Extensive destruction of epidermis and dermis after oral sensitization with 100 mg/kg ALA and 50J/cm^2 (H&E stain, ×150).

particularly sensitive regions of the urothelial cells, whereas with oral administration it is not. Some of the PpIX seen after oral administration may have been synthesized in the liver and reached the bladder via the bloodstream. Nevertheless, this does suggest that intravesical ALA is preferable for management of superficial bladder cancer with PDT, particularly for field change disease.

The substantially higher porphyrin levels (mostly PpIX, but possibly with smaller amounts of uroporphyrins and coproporphyrins [5]) detected in the liver soon after oral ALA are likely to be due to rapid absorption and first pass conversion in the liver. The levels after oral and intravesical sensitization do not become comparable until 7 hours after delivery, although they are still relatively high at this time. Intravesical administration would appear to slow down rather than eliminate systemic absorption as there are still substantial amounts of PpIX detected in the liver, but if the delay in absorption is comparable to the time for PpIX to be metabolized to heme, then the levels in tissues other than the bladder are likely to stay low at all times, reducing the risks of systemic phototoxicity. From our clinical studies, transient elevation of serum aspartate aminotransferases was found in one-third (6/18) of patients receiving 60 mg/kg oral ALA [12]. We did not carry out any enzyme assays in this study, but it seems unlikely that rises would be seen after intravesical administration, although this warrants further studies.

With the kidney, the situation is different. With bladder instillation, in addition to systemic absorption, there is also the possibility of ureteric reflux, which might explain our rather variable results as seen in Figure 5. Clinically, prevention of vesico-ureteral reflux can be achieved by reduction of the intravesical pressure during instillation by reducing the volume of fluid used. We have no evidence at present that increased levels of PpIX in the kidney, both at cellular and subcellular levels, do any harm in the absence of light, but this does need further investigation. Definitive answers can be provided only by examining changes in subcellular structure or the presence of tissue specific substances (such as Tamm Horsfall protein) in the renal tubule or the glomerulus after prolonged contact with ALA and PpIX.

Using fluorescence microscopy for quantitative studies of the tissue distribution of photosensitizers, there is always concern that the results might not be as accurate as using a chemical extraction method. However, the advantage is that fluorescence levels in the various tissue layers can be resolved, whereas extraction techniques only provide an averaged, although absolute, measurement. It could be argued that fluorescence quenching may differ between layers, but previous studies with ALA on rat colon and stomach do not support this contention as microscopic fluorimetry showed good correlation with absolute levels measured separately in the mucosa and muscle [25]. A similar study on the bladder would not be feasible because of the thinness of the urothelium. Further, it may not be reliable to compare the absolute porphyrin levels in different organs solely on the basis of fluorescence microscopy, especially if several different porphyrin species are present. However, using a chemical extraction method, Schoenecker et al. [26] found that the concentration of liver and kidney porphyrins 4 hours after intraperitoneal injection of 200 mg/kg ALA were 35.8±10.1 and 20.1±11.3 μg/mg, respectively. The ratio of liver/kidney concentration of porphyrins was 1.8 (range 1–5.2) in their study, which is roughly comparable to our figure of 2.6 using fluorescence microscopy. Thus it sees likely that the fluorescence measurements are giving reasonably accurate values for the relative levels of porphyrin between organs and between different layers of each organ.

In this study we demonstrated that light alone was not able to induce any detectable skin lesion on macro or microscopic examination. However, the degree of epidermolysis and keratolysis was considerably more prominent in rats receiving oral ALA (100 or 200 mg/kg) than with bladder instillation. Our study on the skin was very limited, but it does suggest that there is less cutaneous photosensitivity giving the ALA intravesically. This is not a major clinical problem, as photosensitivity clears within 1–2 days anyway, but it is another factor in favor of bladder instillation.

Another possible advantage of intravesical ALA for photodynamic management of urinary bladder cancer with field change disease is the seemingly better selectivity achievable between the urothelium and underlying layers over that with oral ALA. The resultant subepithelial fibroblast infiltration 7 days after PDT is apparently more with oral ALA-based PDT. Further evaluation of the bladder at 6 months with VanGieson stain showed more collagen fibrils in the lamina propria after oral ALA sensitization than instillation, although the animals in the oral group were given 400 mg/kg rather than the 100 mg/kg that would have given comparable urothelial levels of PpIX. The actual role of collagen in the bladder is poorly defined, but less collagen deposited in the lamina propria is likely to mean less chance for the bladder to become functionally impaired as a result of altered elasticity.

Management of bladder cancer, unlike most other malignancies, is focused primarily on the prevention of recurrence, which is estimated as high as 40–60% at 12 months after initial treatment of papillary tumors [27]. Measures such as intravesical chemotherapy with antineoplastic agents [28] and Bacillus Calmette-Guerin (BCG) [29] have been tried with some success. However, for effective prevention of recurrence, the best option is to replace the diseased urothelium, which may be in patches or be generalised, with healthy transitional cell lining. PDT of the bladder offers the possibility of achieving this by destroying urothelium without damaging the underlying muscle and repairing the necrosed area with regeneration of normal tissue. Our experiments suggest that PDT with ALA has potential for treating dysplasia of the urothelium, even if it is generalised, and for treating superficial bladder cancers (provided the PpIX reaches the deeper parts of the tumor), or for prevention of recurrence. The preferred route of administration is intravesical as urothelial levels of PpIX are more sustained, systemic effects are less and cutaneous side effects are milder than after oral administration.

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